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Review

P53 as an important diagnostic biomarker in gall bladder neoplasms

Role of P53 in gall bladder neoplasm

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Abstract
Gallbladder carcinoma (GBC) is considered a fatal but seldom occurring cancer of the digestive tract. The process through which a gall bladder adenoma progresses to carcinoma is still unclear. Also, GBCs are seen to appear from pathways comprising metaplasia or dysplasia or from pre-existing adenomas. The only chance of cure is early diagnosis yet despite advances in imaging modalities it seems impossible. Biomarkers are considered an indicator of a biological state. The p53, biomarker lies among rapidly mutated tumor suppressor genes in neoplasms. p53 serves as a scout, which checks for abnormal cell growth against cancer in most of the cells. Proto-oncogenes and the tumor-suppressor genes are considered important factors in the development and prognosis of gallbladder neoplasms. P53 has also been found in gallbladder carcinomas in various studies.

Keywords
Gallbladder Carcinomas, Biomarkers, p53, Molecular Marker, Protein Expression

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Introduction

Gallbladder carcinoma (GBC) is regarded among the most widespread malignancies of the biliary tract. [1] Various regions with the remarkably high incidence include Central and South America (Mexico, Chile, and Bolivia), Japan and Central and Eastern Europe (Hungary, Czechoslovakia, Poland, and Austria). Even with the use of advanced techniques, the survival rate of the patients is low compared to other carcinomas. Also, it has been seen that although the disease has a low incidence, it is usually diagnosed at later stages. [2,3] Preoperative clinical and radiological staging were essential procedures for determining the prognosis of GBC but lately, many practitioners do not find the use of conventional diagnostic methods helpful and they are not able to accurately stage the disease. [4] Characteristics of advanced malignant lesion include localized invasion, distant metastasis, extensive regional lymph node metastasis and vascular encasement due to these features, complete surgical resection is not possible. Hence, the only curative option available is complete resection at an early stage of the disease but only minimal patients report at an early stage. [5] Therefore, here comes the role of a reliable biological marker, which will act as a valuable diagnostic aid, thus helping in facilitating proper treatment plan and providing holistic care to the patients.

Methodology: Various articles from the online sources like Pub Med, MEDLINE, GOOGLE Scholar were studied and evaluated for establishing the role of Biomarker p53 in the carcinoma of Gall bladder.

Cell growth cycle

The trademarks of tumour are unrestricted cell growth and proliferation occurring mainly due to loss of command over cell cycle checkpoints. The group of cyclins, cyclin-dependent kinases (CDKs), and their inhibitors (CDKIs) monitors the cell cycle by the event of activation and inactivation of phosphorylation. [6] The aggregation of numerous genetic variations leads to the development of GBCs, and out of these variations, disruption of cell cycle control is a key character. It often appears as poor regulation of cyclin protein expression, raised activity of cyclin dependent kinases, change in the expression or functioning of cyclin-dependent kinase inhibitors and transformation of cell cycle checkpoint control. [7] Also, the results have shown the involvement of suppressor genes, oncogenes and DNA repair genes, along with changes at molecular levels like epigenetic alterations and microsatellite instability. [8]

Molecular and genetic basis of gallbladder cancer

In the histologic analyses of GBC specimens, a stepwise progression from hyperplasia-atypical hyperplasia, metaplasia-dysplasia, or gallbladder adenomas to carcinoma is seen. Two distinct pathways were suggested related to GBC's.[9] The first one is via adenoma-carcinoma sequence, excluding p53, K-ras or APC gene mutations, and the second pathway is de novo, where there is frequent p53 alteration related to low K-ras mutation percentage. [10] As previously mentioned, activation of cellular genes occurs along with inactivation of tumor suppressor genes that leads to the development of cancer. [11] Molecular studies conducted in this field have shown that there is allelic loss of different chromosomal locations seen in GBC. The results from various studies also suggested the involvement of multiple tumour suppressor genes in the pathogenesis of GBC [12] (Figure 1). Mutations of the p53 gene are genomic aberration that has been detected among approximately 27%–70% of gallbladder carcinomas. [9]

Biomarker in GBC

A 'biomarker' can be described as a biological marker that converts into a state or expression of a protein that is consistent with the possibility of progression of the disorder or with the susceptibility of the disorder for the ongoing treatment. Biomarkers help in providing information regarding healthy and pathologic states along with monitoring the response to therapeutic intervention. When a biomarker establishes its validity, it can be further used for determining the risk of the disease, diagnosing the disease in an individual and to formulate a suitable treatment plan for the patient. Biomarkers can also be categorized as predictive and prognostic markers. A predictive marker is used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent, for example, the Human leukocyte antigen allele (HLA)–B*5701 genotype can be used for determining the human immunodeficiency virus (HIV) patients before abacavir treatment, i.e., to check patients who are at risk for developing serious skin allergies or reaction. Whereas, prognostic markers are used to identify the likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest example Breast Cancer genes 1 and 2 (BRCA1/2) mutations can be used to assess the probability of development of a second breast cancer when evaluating patients with breast cancer. [13]

A tumor marker is a biomarker that is produced by cancer or tumor cells, it can be defined as "a substance expressed either by a tumor or by the host body in response to a tumor and that helps in cancer detection and monitoring". Tumor markers are normal endogenous by-products that are secreted at a higher amount by cancerous cells or are the result of active genes that are at a dormant state in healthy cells. The presence of cancer can be detected through these markers as these are produced in significant amounts by cancerous cells.

Their presence can be marked in the tissues as an intracellular substance or these can be seen in the body serum. Their presence can be measured in various body fluids and in the serum. [14, 16]. (Table 1)

About p53 biomarker

p53 is an antioncogene and its mutation is detected on the short arm 17 encoded as 53-kD nuclear phosphoprotein that acts as an inhibitor of cell proliferation and is also a transcription factor. [17, 18] p53 is a nuclear transcription factor and transactivates various genes that are involved in the initiation of cell cycle arrest and/or apoptosis. Lower levels of p53 are expressed, normally caused by proteasomal degradation mediated majority by RING-finger type E3 ubiquitin protein ligase MDM2, which is a functionally latent form. When DNA damage occurs, p53 gets accumulated in the nucleus of cell via post-translational modifications like acetylation and phosphorylation. [19]

Structure of p53 (Figure 2)

p53 is made of various components including [20]

1. NH2-terminal acidic transactivation domain; N- terminal (NT)

2. DNA-binding domain; core domain and
3. Tetramerization (Tet) domain, and
4. C-terminal (CT) domain

Results

Table 1 depicts various biomarkers involved in Gall bladder carcinoma.

Discussion

Activation of P53 and its pathway

DNA can be altered by various methods like adding an alkyl

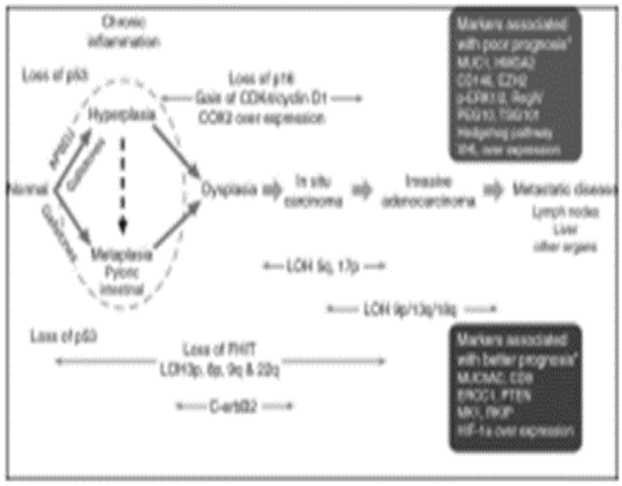

Figure 1. Carcinogenesis at molecular level. [12]

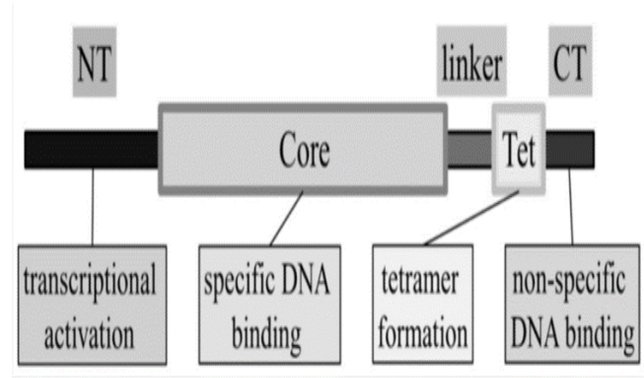

Figure 2. Primary structure of p53 [32]

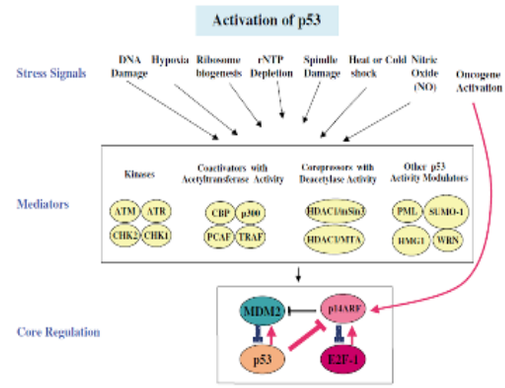

Figure 3. Signals cause activation of p53 that contributes to the primary role of p53 as a suppressor gene [33]

Table 1. Numerous biomarkers determined in the neoplastic conditions of the gall bladder

Marker	Normal	Hyperplasia	Metaplasia	Dysplasia	Carcinoma in situ	Invasive cancer	Reference
MUC2	0%	-	29%	9%	-	11%	21
	-	-	-	75%	100%	58%	22
	-	-	-	75%	-	64%	23
	-	-	91.70%	-	-	-	24
CK20	0%	-	17%	31%	-	18%	21
	0%	100%	100%	-	-	100%	25
	-	-	-	-	-	28.5%	26
	-	-	-	-	-	27%	27
MUC5AG	89%	-	92%	53%	-	38%	21
	21.70%	-	-	60.80%	-	51.90%	28
	-	-	-	85%	-	78%	22
CDX2	-	-	-	-	-	29.2%	29
	-	-	91.7%	-	-	-	24
	5	-	-	-	-	36.8%	30
	-	100%	-	-	-	45.4%	21
MUC6	100%	-	100%	65%	-	27%	21
	-	-	-	80%	90%	91%	22
MUC1	0%	-	0%	35%	-	75%	21
	-	-	-	21.7%	-	57.4%	24
	-	-	-	50%	-	80%	23
	0-20%	-	-	-	-	78-89%	24
CK7	100%	-	100%	100%	-	87%	21
	100%	100%	100%	-	-	100%	25
	-	-	-	-	-	69.5%	26
	-	-	-	-	-	82%	27

group to various bases, loss of purine or pyridine or by oxidative free radicals' reaction. The cells employ a different detection and repair mechanism for the damaging agent. Enzyme activities occur as a reaction to these DNA damages that detect and repair the DNA. Activation of enzyme activities occurs due to DNA damage, causing modification in the p53 protein at the level of amino-acid residue. These enzymes communicate with the p53 protein that damage has been occurred in DNA, hence, post-translational modifications take place that lead to ubiquitination, phosphorylation, methylation, acetylation or sumoylation of the biomarker. On determining the stress posttranslational alteration occurs through the media of these signals, which are transmitted to proteins and processed by a code. [33] (Figure 3)

The alterations in p53 protein caused by protein modifications represent in two ways. In the first way, it is seen that protein's half-life in the cells raises from 6-20 mins to hours resulting in 3-10 folds increase in the protein concentration in a cell. Second, binding capacity of p53 to certain DNA sequences is increased, which advances the transcription of genes modulated by those DNA sequences. Hence the activation of the p53 gene occurs due to these two processes. These stressors cause various changes in the protein that have the potential to disrupt duplication of the cell that further leads to an increase in mutation rate during cell division. The process that is observed and considered to be related with a mutation in the p53 cell is called gene amplification, deletion and aneuploidy. [34] Malkin et al., [35] concluded that the absence of the p53 gene leads to the development of cancer at an early age.

Functions of P53

P53 is responsible for gene expression that is associated with programmed cell death, formation of the new blood vessels, arrest, and pathways through which DNA repair takes place. Also, its role is seen in the starting of the intrinsic apoptotic pathway leading to the arrest of the cell cycle at G1 phase as an outcome to damage caused to DNA. Apoptosis occurs as a result of the activated cellular signalling cascades by p53 protein when DNA damage occurs. Hence, p53 is crucial for sustaining genomic stability and for governing cell growth and proliferation. [36]

Another important role of P53 is conserving genomic integrity. One of the very common changes in tumors in humans are point mutations of cell proliferation in the p53 gene. The majority of these alterations are missense and often causes loss of formation of protein and production of a mutated protein, which displays an increased half-life. [37, 38] Data from various studies has shown that nuclear p53 protein was recognized during immunohistology staining, and these were related with the existence of p53 gene mutation. It was also reported that the correlation of p53 with certain tumors has been poor and there may not have been a necessary gene alteration, hence this can be useful information for an altered protein function of p53. [17]

Role of P53 in gall bladder cancer

One of the earliest events in the pathogenesis of carcinoma of gallbladder is the mutation of p53 gene. It leads to metaplasia-dysplasia-carcinoma succession of GBC. But the role of p53 in GBCs, as a prognostic marker has not yet been established. [6] Alteration of p53 and the aggregation of p53 were observed in around 27% and 70% of gallbladder carcinomas, respectively. Research in patients with GBCs has demonstrated thatmutations in the middle of axons 5 and 8 correspond to deregulation of the p53 gene. [8]

Many authors have studied the overexpression of p53 concerning GBC. Shu et al. [39] in their study found that expression of p53 is closely related to the progression of carcinoma, p53 expression is closely related to carcinogenesis, progresses rapidly, metastasizes easily, is highly invasive, and has a poor prognosis in GBCs. Kim et al [6] also concluded that overexpression of the gene p53 or its loss is poorly associated with the differentiation of the tumor, showing distant metastases and low specific survival rate. Matsubara et al. [40, 41] found around 75% of p53 gene alterations in GBCs and 16.7% and 35.7% of noncancerous lesions in the common bile duct and gallbladder, respectively. Another study concluded the overexpression of the p53 in the noncancerous lesions of the gall bladder. [42] A study by Nagai et al. [43] also represented similar results where they reported alteration of p53 in GBCs. Wang et al. [44] reported a relative increase of around 59.1% in the expression of p53 in the carcinomas of gall bladder and 17.6% (3/17) of gallbladder adenoma cases (p = 0.009). In a study by Neyaz et al. [45] 103 (44.8%) of the included 230 cases showed mutant protein expression in GBC cases.

Conclusions

There is a wide association of p53 seen in gallbladder carcinomas, which is clear from the literature from past to present. A molecular marker acts above the diagnostic marker in certain suspicious cases, and also it acts as a valuable prognostic tool and can help in providing early intervention to conserve the organ. Not only p53, but there are other markers which can aid in early detection and diagnosing in the development of GBSs hence more research literature is needed in this field.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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